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13. ABSTRACT (Maximum 200 words)

We are using protein engineering to produce biomolecular materials that mimic or extend the properties of materials found in nature. Our current focus is on bacterial S layers and the elastomeric protein abductin. For the most part, these studies are in an exploratory phase. In the case of S layers, we are examining the fundamental properties of the SbsB S layer of Bacillus stearothermophilus by structure-function studies using site-directed mutagenesis and targeted chemical modification. The major finding has been the delineation of a mechanism for the in vitro formation of S layers from their SbsB building blocks. Applications of S layers in biotechnology are also being investigated in parallel with these studies and an extensive cysteine-scanning mutagenesis has been performed to determine sites at which the protein can be chemically modified. In addition, in collaboration with the group of Uwe Sleytr (Vienna), we are using S layers to support bilayers containing genetically engineered pore-forming proteins. These materials will serve as rugged biosensor elements. In the case of abductin, a major advance has been the determination of the sequences of cDNAs encoding the protein.

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MOLECULAR GENETIC APPROACHES TO BIOMOLECULAR MATERIALS

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OCTOBER 16, 2000

U. S. ARMY RESEARCH OFFICE

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b. Final Progress Report

(1) Foreword.- NA

(2) Table of contents- NA

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(4) Statement of the problem studied

We are using protein engineering to produce biomolecular materials that mimic or extend the properties of materials found in nature. Our current focus is on bacterial S layers and the molluscan protein abductin. S layers form the outer envelopes of many bacteria. As two-dimensional protein crystals, they are expected to have many interesting applications in biotechnology. Abductin is the major protein constituent of the highly elastic inner hinge ligament of the scallop. The protein, or recombinant derivative of it, will form a basis for the preparations of materials with unusual physical properties.

5. Summary of most important results

1) Elucidation of an in vitro assembly pathway for the S layer formed by the SbsB protein of Bacillus stereothermophilus. Assembly occurs when the monomeric protein is above a critical concentration, by a nucleation dependent mechanism (Y. Wang).

2) Exploration of the domains of SbsB required for S layer assembly by using truncation and deletion mutagenesis. The C terminus is critical for assembly (Y. Wang).

3) Development of an improved protocol for high throughput cysteine scanning mutagenesis by PCR and in vivo recombination (S. Howorka).

4) Extensive cysteine mutagenesis in preparation for exploring and modifying the properties of SbsB by targeted chemical modification. Surface accessible residues in the monomeric and assembled forms of the bacterial surface layer protein were revealed by modification with a reactive polymer. The identification of residues crucial for assembly was in agreement with and extended the findings from truncation and deletion mutagenesis. (S. Howorka).

5) Lipid bilayers supported with S layers. S layers were used to support and thereby strengthen conventional lipid bilayers. Further, S-layer ultrafiltration membranes were developed, providing an even more robust support for bilayers (with B. Schuster in the laboratory of U. Sleytr).

6) Sequences of cDNAs encoding the protein component, abductin, of the inner hinge ligament of the mollusk Argopecten irradians. Abductin is the major protein constituent of the highly elastic inner hinge ligament. The unusual sequence of the protein was previously unknown and contains a Gly-Gly-Phe-Gly-Gly-Met-Gly-Gly-Gly-Xaa repeat (Q. Cao).

7) Visualization of the α-hemolysin pore and an assembly intermediate by atomic force microscopy. This worked confirmed the heptameric structure of the pore under a variety of conditions. It also showed that the prepore assembly intermediate is a

heptamer with its seven-fold axis perpendicular to the membrane surface (ARO project of J. Yang).

8) Purification and characterization of recombinant spider silk expressed in *Escherichia coli* . Genes encoding a spider silk repeat sequence were prepared and expressed in *Escherichia coli* . Sufficient material is obtained for studies of fiber processing (with the laboratory of D. Kaplan)

6. List of manuscripts published under ARO sponsorship during this reporting period

Papers:

1) Fang, Y., Cheley, S., Bayley, H., and Yang, J. The heptameric prepore of staphylococcal α-hemolysin mutant in lipid bilayers imaged by atomic force microscopy. **Biochemistry** 36, 9518-9522 (1997)

2) Cao, Q., Wang, Y., and Bayley, H. Sequence of abductin, the molluscan "rubber" protein. **Current Biology**, 7, R677- R678(1997)

3) Cheley, S., Malghani, M.S., Song, L., Gouaux, J.E., Yang, J., and Bayley, H. Spontaneous oligomerization of a staphylococcal α-hemolysin conformationally constrained by removal of residues that form the transmembrane β-barrel. **Protein Engineering**, 10, 1433-1443 (1997).

4) Arcidiacono, S., Mello, C., Kaplan, D., Cheley, S., and Bayley, H. Purification and characterization of recombinant spider silk expressed in Escherichia coli. **Appl. Microbiol. & Biotechnol.** 49, 31-38 (1998).

5) Schuster, B., Pum, D., Braha, O., Bayley, H., and Sleytr, U.B. Self-assembled α-hemolysin pores in an S-layer supported lipid membrane. **Biochim. Biophys. Acta** 1370 280-288 (1998)

6) Howorka, S., and Bayley, H. Improved protocol for high throughput cysteine scanning mutagenesis, **Biotechniques** 25, 764-772 (1998).

7) Howorka, S., Sára, M., Wang, Y., Kuen, B., Sleytr, U.B., Lubitz, W. and Bayley, H. Surface accessible residues in the monomeric and assembled forms of a bacterial surface layer protein. **J. Biol. Chem.**, in press (2000)

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9) Wang, Y., Howorka, S., Kuen, B., Lubitz, W., Sleytr, U.B. and Bayley, H. In vitro assembly pathway of the S layer formed by recombinant and native Bacillus stearothermophilus PV72 SbsB protein, in preparation

10) Wang, Y., Howorka, S., Kuen, B., Lubitz, W. Sleytr, U.B. and Bayley, H. SbsB protein of Bacillus stearothermophilus: effects of truncation on S layer formation, in preparation

Hagan Bayley	Professor and Head		
Oxford University, Balliol College	BA (1st)	1974	Chemistry
Harvard University	Ph.D.	1979	Chemistry
Massachusetts Institute of Technology	Postdoc.	1979-1981	Chemistry & Biology

RESEARCH & PROFESSIONAL EXPERIENCE

Professor and Head, Medical Biochemistry and Genetics, Texas A&M University Health Science Center, Professor of Chemistry, 1997-present

Principal Scientist, Worcester Foundation for Biomedical Research, (1994-1996); Senior Scientist, (1988-1994); Associate Professor of Biochemistry & Molecular Biology, University of Massachusetts Medical Center (1991-1996); Associate Professor of Chemistry, Clark University (1996)

Associate Professor, Center for Neurobiology & Behavior, Columbia University (1987-88), and Assistant Investigator, Howard Hughes Medical Institute, Columbia University (1985-88)

University Lecturer in Organic Chemistry, Oxford University (1984-85), and Fellow of Brasenose College, Oxford (1984-85)

Assistant Professor of Biochemistry (1981-84), Columbia University

AWARDS

1970: Open scholarship, Oxford University; 1972: Distinction in Chemical Pharmacology, Oxford University; 1973: Hebertson Prize for Chemistry, Balliol College, Oxford; Gibbs Prize for Chemistry, Oxford University; 1983: Irma T. Hirschl Career Scientist Award.

RESEARCH PROJECTS ONGOING OR COMPLETED DURING THE LAST 3 YEARS

Assembly, structure and function of pore-forming proteins
Principal Investigator; Hagan Bayley, PhD
Present support: DOE (6/15/00-6/14/03), renewal $480,000
The original goal of this project was to understand the assembly pathway and structure of a pore-forming bacterial toxin, α-hemolysin. Significant progress has been made and at a descriptive level the problem has been to a large extent resolved. Our current efforts have been redirected towards the redesign of α-hemolysin, and the de novo design and *in vitro* evolution of β- barrel membrane proteins. This project uses only molecular genetic approaches, rather than chemical modification, and is therefore distinct from the work proposed here.

Applications of engineered pore-forming proteins
Principal Investigator: Hagan Bayley, PhD
Present support: University of Texas at Austin, Air Force-Multi-site program (6/1/98-11/30/01) $368,350
Present support: Texas Advanced Technology Program -1999 (1/1/00-12/31/01) $159,076
Present support: MURI (ONR) - Multisite program project (4/30/99-4/29/04) $635,073
Pore-forming proteins are being engineered for applications in biotechnology. Our main focus is on the pore-forming bacterial toxin, α-hemolysin. We have used protein engineering to make modified hemolysins with built-in triggers and switches. Pore formation can then be actuated or modulated by biochemical stimuli (e.g. enzyme action), chemical stimuli (e.g. the association and dissociation of metal ions), and physical stimuli (e.g. light). Recent efforts have been directed at using engineered hemolysins as elements in biosensors. New directions include optical and microwave signal detection. Additionally, we have explored the use of engineered hemolysins for the controlled permeabilization of cells, drug delivery and the destruction of malignant cells.

Caged peptides and proteins for signal transaction research
Principal Investigator: Hagan Bayley, PhD
Present support: Welch Foundation (6/1/00-5/31/03) $135,000
The use of "caged" reagents allows the photogeneration of molecules on or in cells with precise spatial and temporal control. In signal transduction research, effectors and inhibitors can be released at known sites, in defined doses, and at predetermined times. We are using a variety of photoremovable protecting groups to cage peptides and proteins for studies of cell signaling. One tactic we have used has been to dervatize proteins engineered to contain single cysteines at key positions. The activities of many cell signaling proteins are modulated by phosphorylation. Therefore we are also examining peptides and proteins modified on the sulfur atom of thiophosphoryl groups. We are seeking ways other than microinjection to transfer caged proteins to the cell interior based on our knowledge of membrane protein assembly.

Membrane protein engineering by targeted modification
Principal Investigator: Hagan Bayley, PhD
Present support: NIH (3/15/00-2/28/04) $1,620,000
The properties of α-hemolysin are being re-engineered by using targeted chemical modification. The work differs from the DOE project, which employs direct genetic modification. Targeted modifications include covalent attachment of chelating agents and polymers, as well as non-covalent modification with molecular adapters such as cyclodextrins and cyclic peptides.

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PATENTS (of seven):

Stochastic sensing mediated by carrier molecules (November 1998, provisional patent filed; November 1999, full patent filed)

Biosensor compositions and methods of use (February 200, provisional patent filed)